

INFLUENCE OF VASOACTIVE DRUGS ON BLOOD FLOW IN SUBCUTANEOUS  
TUMORS - AN EXPERIMENTAL STUDY IN RATS.

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## ABSTRACT

As a model for the study of metastatic tumor blood flow and how it is affected by vasoactive drugs subcutaneously implanted tumors in Wistar and Lister rats were used. Blood flow measurements were made using the labeled microsphere method and the reference organ technique. 15  $\mu$ m microspheres, labeled with either  $^{99}\text{Tc}^m$  or  $^{51}\text{Cr}$  isotopes, were injected intracardially and simultaneously a reference sample was drawn at constant speed. This was performed before and after the infusion of vasoactive drugs, and as control, saline infusion. The drugs tested (adrenaline, noradrenaline, glucagon, histamine and vasopressin) showed profound effects on central hemodynamic parameters and on muscle and skin blood flow. The relative tumor blood flow measured as the ratio between tumor and skin or tumor and muscle blood flow was calculated. The results show that no enhancement of the relative tumor blood flow could be registered. In fact, a decrease in the tumor to muscle blood flow ratio was noted when noradrenaline was infused and in the tumor to skin ratio when adrenaline was infused. Blood flow in subcutaneous tumors was 4 - 5 times that of muscle or skin. Tumor blood flow was also inversely related to the size of the tumor.



## INTRODUCTION

The blood supply of malignant tumors may play an important role in the treatment of cancer. Most experimental and clinical work in this field has been projected against liver tumors, e.g., liver dearterialization, regional intraarterial infusion of cytostatic drugs (2, 3, 6, 25).

It is reasonable to presume that also in tumors with other localization than the liver the vascular supply influences the results of cytostatic treatment and radiotherapy (15).

Hyperthermia as a part of the multimodel treatment of cancer together with irradiation or surgery is gaining increasing interest (14). For hyperthermia the blood flow of the tumors might also be of considerable importance.

The blood flow in experimental tumors is dependent on the size of the tumor. In small tumors the blood flow is relatively larger than in big tumors and much larger in the periphery of the tumor than in the central parts (8, 26). When the tumor has reached a certain size, the central part becomes necrotic because of insufficient nutrition. This central part of the tumor is rather uninteresting from the blood flow point of view. On the other hand, in small tumors with a large growing compartment and an active circulation, it might be possible to influence the blood flow - reduce to initiate necrosis, or increase to conduct more cytostatic to the proliferative parts of the tumor. This might be of therapeutic value. Blood flow in liver tumors can be influenced by vasoactive drugs; noradrenaline and vasopressin have been investigated by the present authors and a relative increase of blood flow of the tumor compared to the surrounding liver parenchyma was demonstrated after noradrenaline infusion, vasopressin, on the other hand, caused a diminished relative arterial perfusion of the liver tumors (12, 13).

The blood flow in tumors can be measured in several ways, e.g., by studying the wash-out curves of inert gases (Xe-133, Kr-85) or using radioactively labeled vehicles like rubidium-86, etc (10, 21, 23, 28). We, and others, have found labeled microspheres using the reference organ principle easy to handle and to provide good reproducibility (4, 5, 7, 11).

The present study aims to establish the blood flow in subcutaneous tumors measured by labeled microspheres by means of the reference sample method and also to study the effect of infused vasoactive drugs on tumor blood flow. A model for the investigation of distant metastatic tumors from primary cancer has been set up with subcutaneously implanted tumors in the rat.



## MATERIAL AND METHODS

Fifty-eight inbred rats of Wistar and Lister strains with an average weight of  $231 \pm 5$  g were used. The rats were inoculated with a standardized tumor cell suspension containing approximately 500 000 cells/ml on the dorsal aspect of the rats in two locations. Wistar rats were inoculated with an NG-W adenocarcinoma and the Lister rats with an Hep-H hepatoma. Tumor take was approximately 80 %. About 12 days after the tumor inoculation the blood flow study was performed. The tumor diameter was about 10 mm and tumor weight was  $0.80 \pm 0.07$  g.

The rats were anesthetized with sodium pentobarbital (30 mg/kg bodyweight intraperitoneally). One catheter was introduced via the right carotid artery into the left ventricle of the heart, one via the left femoral artery into the lower abdominal aorta and one via the right jugular vein to the superior subclavian vein. The latter catheter was used for infusion of the drugs and the aortic catheter for suctioning the reference sample by means of a Harvard type withdrawal pump. The catheter in the left ventricle was used for infusion of microspheres and also for measuring systolic intraventricular pressure and heart rate. This was recorded throughout the experiment using an Elema-Schönander EMT 34 Transducer.

Tracer Sephadex<sup>R</sup> microspheres, consisting of cross-linked dextran (Pharmacia Fine Chemicals AB, Uppsala, Sweden) with a diameter of 15  $\mu$ m were used. They were labeled with either  $^{99}\text{Tc}^{\text{m}}$  or  $^{51}\text{Cr}$  isotopes and dissolved in Ficoll 70<sup>R</sup> solution. The nominal activities were 25 - 70  $\mu$ Ci for the  $^{99}\text{Tc}^{\text{m}}$  and 12 - 14  $\mu$ Ci for the  $^{51}\text{Cr}$  isotope. Approximately 800 000 - 1 000 000 microspheres dissolved in 0.2 ml saline were agitated vigorously and injected into the left ventricle. The catheter was then flushed with 0.5 ml saline. The total time for the injection procedure was about 15 - 20 sec. Just prior to the injection of the microspheres the reference sample withdrawal pump was started, drawing the reference sample at a speed of 0.5 ml/min. This pump was stopped after about 40 - 60 sec. After infusion of the vasoactive drug and when the animal had reached a new circulatory steady state the second injection of microspheres labeled with the other isotope was performed and the second reference sample was drawn. Reference samples and tissues from skin and muscle as well as tumor tissues were taken, weighed and counted in a two-channel well-type scintillation counter. The  $^{99}\text{Tc}^{\text{m}}$  isotope was measured at 140 keV and the  $^{51}\text{Cr}$  isotope at 324 keV. A computerized program designed for the experiment was used for the calculations. Cardiac output is expressed in  $\text{ml} \cdot \text{min}^{-1} \cdot 100 \text{ g}^{-1}$  bodyweight and tumor and tissue blood flow in  $\text{ml} \cdot \text{min}^{-1} \cdot \text{g}^{-1}$ .



The rats were divided into one control group infused with physiological saline at a rate of 0.2 ml/min to a total volume of 1.0 ml and 5 experimental groups receiving the active drugs which were adrenaline, noradrenaline, glucagon, histamine and vasopressin (Table I). Infusion rate was 0.2 ml/min.

TABLE I Vasoactive drug infusion study in rats with subcutaneous tumors.

| Group | Drug                   | Number<br>of rats<br>n | Dosage        | Number<br>of tumors<br>n | Tumor<br>size<br>g |
|-------|------------------------|------------------------|---------------|--------------------------|--------------------|
| A     | Control (Phys. Saline) | 7                      | 0.2 ml/min    | 14                       | 0.51 $\pm$ 0.08    |
| B     | Adrenaline             | 12                     | 4 ug/min/kg   | 14                       | 0.99 $\pm$ 0.21    |
| C     | Noradrenaline          | 11                     | 5 ug/min/kg   | 20                       | 0.99 $\pm$ 0.19    |
| D     | Glucagon               | 9                      | 4 ug/min/kg   | 17                       | 0.60 $\pm$ 0.11    |
| E     | Histamine              | 9                      | 2 ug/min/kg   | 16                       | 0.73 $\pm$ 0.18    |
| F     | Vasopressin            | 10                     | 0.04 U/min/kg | 11                       | 0.92 $\pm$ 0.26    |
|       |                        | 58                     |               | 92                       | 0.80 $\pm$ 0.07    |

Blood flow in tumors as well as the ratio between tumor and muscle or skin blood flow was calculated. For statistical analysis results are presented as mean  $\pm$  standard error of mean (SEM). Comparisons between groups are made by means of Student's T-test for paired and unpaired observations. The results of the two strains are presented together but when varying results between these two subgroups were obtained this is reported.

## RESULTS

The largest (more than 1 cm in diameter) subcutaneous tumors showed signs of central necrosis. The effects on systolic intraventricular pressure, heart rate and cardiac output of the drugs on the six groups are summarized in Table II. The controls showed no effect in any of the parameters. Adrenaline produced a significant increase in blood pressure. Noradrenaline induced a significant increase in blood pressure and decrease in cardiac output. Glucagon decreased blood pressure. Histamine induced no changes. Vasopressin infusion increased blood pressure and decreased heart rate and cardiac output. When analysed separately, Lister and Wistar subgroups showed the same pattern except for the effects on cardiac output where Lister rats after adrenaline infusion showed a significant decrease whereas the Wistar rats were not affected.

TABLE II

Intraventricular systolic pressure, heart rate and cardiac output before and after infusion of drugs (mean  $\pm$  SEM). Paired T-tests.

| Group      | Systolic pressure<br>mm Hg |                            | Heart rate<br>beats $\times$ min <sup>-1</sup> |                             | Cardiac output<br>ml $\times$ min <sup>-1</sup> $\times$ 100 g <sup>-1</sup> |                              |
|------------|----------------------------|----------------------------|------------------------------------------------|-----------------------------|------------------------------------------------------------------------------|------------------------------|
|            | Before                     | After                      | Before                                         | After                       | Before                                                                       | After                        |
| A          | 129 $\pm$ 12               | 125 $\pm$ 8                | 364 $\pm$ 9                                    | 360 $\pm$ 8                 | 39.6 $\pm$ 2.8                                                               | 39.8 $\pm$ 4.0               |
| B          | 98 $\pm$ 8                 | 131 $\pm$ 6 <sup>xxx</sup> | 333 $\pm$ 6                                    | 346 $\pm$ 7                 | 32.1 $\pm$ 3.7                                                               | 26.0 $\pm$ 2.8               |
| C          | 104 $\pm$ 8                | 134 $\pm$ 6 <sup>xxx</sup> | 362 $\pm$ 3                                    | 360 $\pm$ 5                 | 31.1 $\pm$ 2.3                                                               | 23.9 $\pm$ 3.0 <sup>xx</sup> |
| D          | 112 $\pm$ 6                | 92 $\pm$ 6 <sup>xxx</sup>  | 360 $\pm$ 7                                    | 362 $\pm$ 7                 | 30.3 $\pm$ 6.1                                                               | 30.3 $\pm$ 4.1               |
| E          | 116 $\pm$ 8                | 117 $\pm$ 7                | 347 $\pm$ 12                                   | 346 $\pm$ 10                | 30.1 $\pm$ 5.7                                                               | 30.6 $\pm$ 8.3               |
| F          | 113 $\pm$ 9                | 138 $\pm$ 8 <sup>xx</sup>  | 341 $\pm$ 10                                   | 308 $\pm$ 10 <sup>xxx</sup> | 22.9 $\pm$ 2.3                                                               | 11.0 $\pm$ 2.2 <sup>xx</sup> |
| Total (58) | 110 $\pm$ ...              |                            | 350 $\pm$ 6                                    |                             | 30.6 $\pm$ 2.1                                                               |                              |

x  $p < 0.05$   
 xx  $p < 0.01$   
 xxx  $p < 0.001$

TABLE III

Alterations in blood flow of skin and muscle tissue induced by different drugs (mean  $\pm$  SEM). Paired T-tests.

| Group<br>(n) | Muscle<br>ml $\times$ min <sup>-1</sup> $\times$ g <sup>-1</sup> |                               | Skin<br>ml $\times$ min <sup>-1</sup> $\times$ g <sup>-1</sup> |                                |
|--------------|------------------------------------------------------------------|-------------------------------|----------------------------------------------------------------|--------------------------------|
|              | Before                                                           | After                         | Before                                                         | After                          |
| A (7)        | 0.15 $\pm$ 0.02                                                  | 0.14 $\pm$ 0.02               | 0.08 $\pm$ 0.02                                                | 0.05 $\pm$ 0.01                |
| B (12)       | 0.11 $\pm$ 0.02                                                  | 0.07 $\pm$ 0.02 <sup>x</sup>  | 0.07 $\pm$ 0.01                                                | 0.03 $\pm$ 0.01 <sup>xx</sup>  |
| C (11)       | 0.13 $\pm$ 0.03                                                  | 0.06 $\pm$ 0.01 <sup>x</sup>  | 0.08 $\pm$ 0.02                                                | 0.04 $\pm$ 0.01 <sup>xxx</sup> |
| D (9)        | 0.11 $\pm$ 0.05                                                  | 0.07 $\pm$ 0.02               | 0.12 $\pm$ 0.06                                                | 0.07 $\pm$ 0.03                |
| E (9)        | 0.11 $\pm$ 0.02                                                  | 0.08 $\pm$ 0.01 <sup>x</sup>  | 0.08 $\pm$ 0.02                                                | 0.05 $\pm$ 0.01 <sup>xx</sup>  |
| F (10)       | 0.10 $\pm$ 0.02                                                  | 0.03 $\pm$ 0.01 <sup>xx</sup> | 0.06 $\pm$ 0.02                                                | 0.01 $\pm$ 0.003 <sup>xx</sup> |
| Total (58)   | 0.12 $\pm$ 0.01                                                  |                               | 0.08 $\pm$ 0.01                                                |                                |

x  $p < 0.05$   
 xx  $p < 0.01$   
 xxx  $p < 0.001$



The effects of different drug infusions on blood flow in skin and muscle tissues are summarized in Table III, which shows that there was no effect of saline or glucagon infusion. Adrenaline, noradrenaline, histamine and vasopressin induced a significant decrease of both muscle and skin blood flow. Average blood flow in 92 subcutaneous tumors was  $0.27 \pm 0.02$   $\text{ml} \cdot \text{min}^{-1} \cdot \text{g}^{-1}$ . Blood flow of subcutaneous tumors in relation to size can be seen in Fig 1. The figure shows a decreased flow in relation to increasing size.

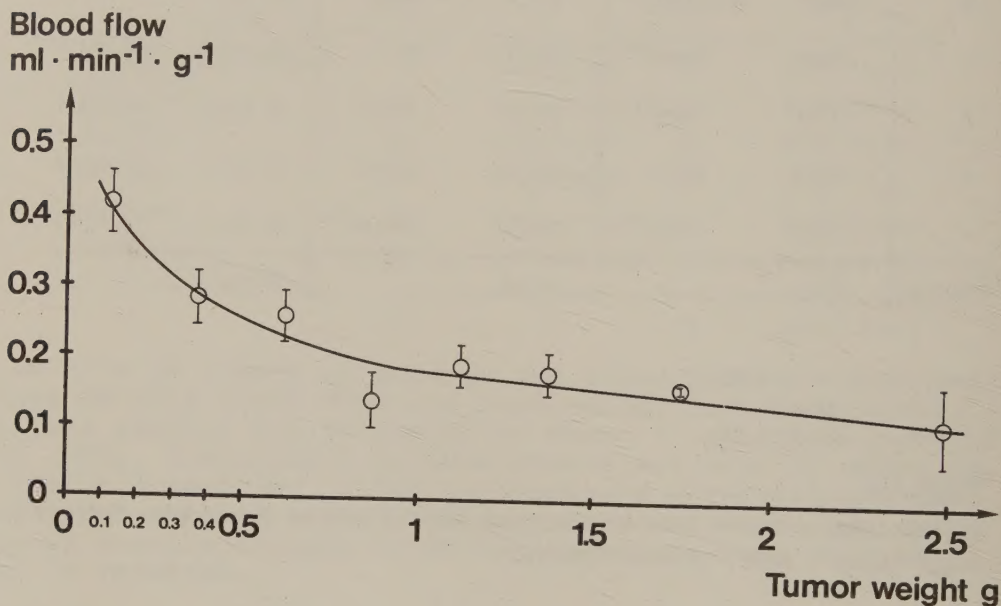


Fig 1. Blood flow in subcutaneous tumors plotted against tumor weight. Each circle represents the mean  $\pm$  SEM based on several tumors in that weight range.

The average blood flow in the tumor in relation to muscle tissue shows a ratio of  $4.0 \pm 0.4$  before infusion. Infusion of the different drugs did not demonstrate any significant changes except for the group infused with noradrenaline, which showed a significant decrease (Table IV). Tumor to skin ratio was  $5.1 \pm 0.9$  before infusion and no significant change was observed after infusion of the different drugs except for the adrenaline infused group which had a significant decrease of this ratio (Table IV). There were no differences in the two subgroups when they were analysed separately. The tumor to muscle blood flow ratio in relation to size showed no definite trend and no changes of that ratio occurred after infusion.



TABLE 1V

Effects on tumor to muscle blood flow ratio and tumor to skin blood flow ratio after infusion of drugs (mean  $\pm$  SEM). Paired T-tests.

| Group | T/M ratio     |                            | T/S ratio     |                             |
|-------|---------------|----------------------------|---------------|-----------------------------|
|       | Before        | After                      | Before        | After                       |
| A     | 3.2 $\pm$ 0.5 | 3.1 $\pm$ 0.5              | 6.2 $\pm$ 1.0 | 8.7 $\pm$ 2.2               |
| B     | 2.2 $\pm$ 0.6 | 1.4 $\pm$ 0.5              | 3.2 $\pm$ 0.6 | 1.4 $\pm$ 0.2 <sup>xx</sup> |
| C     | 3.6 $\pm$ 1.0 | 1.7 $\pm$ 0.4 <sup>x</sup> | 4.0 $\pm$ 0.9 | 3.7 $\pm$ 0.9               |
| D     | 8.0 $\pm$ 3.1 | 4.5 $\pm$ 1.2              | 6.7 $\pm$ 1.8 | 5.0 $\pm$ 0.9               |
| E     | 3.6 $\pm$ 0.8 | 4.3 $\pm$ 0.8              | 5.7 $\pm$ 1.5 | 7.9 $\pm$ 2.2               |
| F     | 2.6 $\pm$ 1.0 | 1.2 $\pm$ 0.4              | 4.5 $\pm$ 1.2 | 3.4 $\pm$ 1.1               |
| Total | 4.0 $\pm$ 0.4 |                            | 5.0 $\pm$ 0.9 |                             |

x  $p < 0.05$

xx  $p < 0.01$

## DISCUSSION

The treatment of generalized malignant disease or locally advanced cancer, especially regarding cancer originating from the gastrointestinal tract, has not made great progress in the last years, although aggressive treatment with multiple cytostatic drugs can provide remission rates in the order of 30 - 40 % (9, 18, 24). The remissions are often of very short duration and the final outcome of the disease is always fatal. In order to increase effectiveness, various ways of administering the cytostatic drugs have been employed, e.g., arterial infusion or isolated regional perfusion. These types of administration give better remission rates and probably also improvement in survival (3, 25, 27).

Another possibility to increase the effectiveness of cytostatic treatment could be the use of vasoactive drugs as an additive therapy. This increase could be reached by redistribri-

buting blood from normal tissues to tumor tissues by using vasoconstrictors that act on normal but not on tumor vessels.

Blood flow measurements in small animals are usually combined with great technical problems and poor reproducibility. The present technique using an artificial reference organ and microspheres labeled with different isotopes has been used by the present group and others, and the theoretical basis for this technique and its difficulties have been extensively evaluated (4, 5, 7, 11, 22). To achieve high accuracy in small tissue samples with low blood flow, a great number of microspheres must be injected in each bolus (7).

In the present experimental model for metastatic subcutaneous tumors the results have shown a rather low blood flow and an inverse relationship of blood flow to tumor size. This has previously been found by Cataland and co-workers (8) and also by us in a previous report (26). If the tumor blood flow is related to blood flow in surrounding tissues, e.g., muscle or skin, a relatively higher perfusion of the tumors seems to exist. When tumor size is around 0.8 g, tumor to muscle or tumor to skin blood flow ratios are in the order of 4 - 5.

It has previously been shown that malignant vessels in liver tumors have no adrenergic innervation and that the tumor vessel walls lack smooth muscles (12, 16, 17). If this is true, an effect of adrenergic vasoactive drugs should be expected because of a difference in reaction to these drugs between tumor vessels and the vessels of surrounding normal tissues. The resulting effect should be a preferential blood flow to the tumor.

In our study on subcutaneous tumors, adrenaline and noradrenaline produced a change in the ratio between tumor to surrounding tissue blood flow. The resulting decrease indicated that these drugs constricted the tumor vessels more than the vessels of muscle or skin tissue. This is contradictory to the statement above but can be explained by the fact that a more pronounced constriction takes place in extratumoral vessels supplying the tumor than in the intratumoral vessels. This decrease is also in contrast to the findings of the behaviour in the liver where an increase of tumor to liver blood flow ratio was noted after noradrenaline infusion (12). The difference might be explained by the fact that in the liver, multiple vessels grow into the tumor from surrounding tissues whereas in subcutaneous tumors one, or only a few arteries supply the tumor (19, 29).

All other tested drugs except for saline and glucagon decreased the blood flow through muscles and skin and as tumor to skin or muscle ratio were unchanged, subsequently tumor blood flow also decreased. This decrease is evidently, due



to peripheral actions of the drugs as significant effects on cardiac output were only noted in the noradrenaline and vasopressin infused rats. Histamine does not affect rat central circulatory parameters, and this was verified in our study. On the other hand, muscle and skin blood flow was reduced and as the tumor to muscle or tumor to skin ratios were unaffected, the perfusion of the subcutaneous tumor was subsequently reduced, a finding which is somewhat contradictory to others where an increased vascular permeability caused by histamine has been noted (1).

To summarize, this study shows that blood flow in subcutaneous tumors is relatively greater than in surrounding skin or muscle tissue. The relative blood flow decreases with an increasing size of the tumor. The five tested vasoactive drugs showed only slight effects on the tumor to skin or tumor to muscle blood flow ratio. In fact there was only a decrease demonstrated after adrenaline and noradrenaline infusion. This study gives no evidence of enhancement of tumor blood flow after infusion of these vasoactive drugs and thus they are of little or no value as additive agents to cytostatic drug therapy.

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